MODEL: (3M532) Subcutaneously-Implanted M5076 Ascitic Sarcoma - Life Span

Origin of Tumor Line: Arose spontaneously in a C57BL mouse, discovered as a mass in the area of the ovary at necropsy, at the Papanicolaou Research Institute, Miami, Florida, in the laboratory of Dr. W. F. Dunning.

Summary of Test Procedures: 5×10^6 cells of ascitic fluid are implanted s.c. in mice. I.P. test agent treatment starts one day after tumor implant and continues every fourth day for a total of four injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

ANIMALS: (refer to Protocol 8)

Propagation: C57BL/6 female mice (intraperitoneal implants).

Testing: $B_6C_3F_1$ mice (male or female).

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all tests, titrations, and control animals in one experiment.

Source: One source for all animals in one experiment. Exceptions must be noted as comments.

EXPERIMENT SIZE: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: A minimum of 40 control animals must be used; otherwise the number of control animals varies according to the number of test groups.

Titrations: Each control is to include titrations of 5×10^6 to 5×10^3 cells, inclusive, with ten animals per level.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6)

Propagation:

Tissue:

Suspension: Prepare 0.1 ml of diluted ascitic fluid containing 1×10^6 cells. (Refer to the following section entitled Preparation of Cell Suspension).

Time: Days 14-19

Site: Implant i.p. 0.1 ml of suspension.

Testing:

Tissue:

Suspension: Prepare 0.5 ml of diluted ascitic fluid containing 5×10^6 cells. (Refer to the following section entitled Preparation of Cell Suspension).

Time: Days 14-19

Site: Implant s.c. 0.5 ml of diluted ascitic fluid using an 0.5 inch, 23 gauge needle with a 3 ml Luer-Lok syringe. Site of injection should be near the axillary region as for trocar fragments.

PREPARATION Of CELL SUSPENSION:

Use donor tumor when the abdomen becomes distended (usually Days 14-19).

Using a sterile 5 ml syringe, withdraw ascitic fluid asceptically through the abdominal wall from which the skin has been removed. Use a minimum of 3 mice. A comment must be provided if more than 20 donor animals are required for an average (330 mice) experiment. Collect at least 15 ml of ascitic fluid. Pool fluid in a sterile glass container held in an ice bath.

Cell Count: Use physiological saline for dilutions. Use serological pipettes with rubber bulb attachment. Cell suspensions should be swirled and mixed by aspirating the solution into and out of the pipette several times before withdrawing an aliquot.

Dilutions:

Suspension A: 0.5 ml of pooled ascitic fluid plus 4.5 ml of physiological saline.

Suspension B: 0.5 ml of Suspension A plus 4.5 ml of physiological saline.

Suspension C: 1.0 ml of Suspension B plus 4.0 ml of physiological saline. Suspension C is a 1:500 dilution and should be used to make the cell count as follows:

1. Agitate Suspension C and fill a white blood cell pipette.
2. Agitate and allow 3 drops to flow out, then fill both chambers of the hemacytometer.
3. Count only intact nucleated cells using 100x to 400x magnification.
4. Assuming the use of an AO STD or comparable hemacytometer, count 4 large squares in both chambers, being sure to establish and follow a convention for inclusion of cells that fall on lines.
5. The cell count and the dilution ratio may be read directly from the accompanying table. To use the table, divide the total number of cells in both chambers (refer to Step 4) by 2.

These values may also be calculated directly from the numbers of cells in both hemacytometer chambers as follows:

$$\frac{\text{Total No. of Cells in Both Hemacytometer Chambers}}{2} \times 2.5 \times 500 \times 1000^{**} = \text{Cells/ml of the Undiluted Ascitic Fluid}$$

Note: Once the cell count has been determined, there is no further need for Suspensions A, B, or C. The cell suspension for inoculation will be made from the original undiluted ascites from which Suspension A was made.

**2.5: Correction factor to convert count to cells per cubic mm.
500: Dilution factor.
1000: Converts mm to ml.

The dilution factors required to prepare a suspension of cells that contain the highest cell number required in 0.5 ml may be calculated as shown in the following example:

$$\frac{\begin{array}{c}10{,}000{,}000\ (\text{Cells/ml}\\ \text{Required for } 5 \times 10^6\\ \text{in 0.5 ml Implant})\end{array}}{\begin{array}{c}120{,}000{,}000\ (\text{Cell Count/}\\ \text{ml of Ascitic Fluid})\end{array}} = \frac{X\ \text{ml (ascites)}}{10\ \text{ml (total volume)}}$$

$$120X = 100$$
$$X = 0.83\ \text{ml}$$

Add 0.83 ml of ascitic fluid to 9.17 ml of physiological saline for a suspension of 5×10^6 cells in 0.5 ml.

Inoculation: Sterile procedures should be followed to dilute the ascitic fluid to obtain Suspension 1 and subsequent dilutions. Cell suspensions must be swirled and mixed by aspirating the solution into and out of the pipette several times before withdrawing an aliquot.

Suspension 1: Highest level required; 5×10^6 cells in 0.5 ml.

Suspension 2: Add 1 part of Suspenion 1 to 9 parts of physiological saline 5×10^5 cells in 0.5 ml.

Suspension 3: Add 1 part of Suspension 2 to 9 parts of physiological saline = 5×10^4 cells in 0.5 ml.

Suspension 4: Add 1 part of Suspension 3 to 9 parts of physiological saline = 5×10^3 cells in 0.5 ml.

Use sufficient numbers of sterile syringes and needles so that that no syringe will be refilled from the pool of donor fluid. No more than 60 minutes should elapse from the time fluid is taken from the donor until it is implanted in the recipient animals. For titrations, inoculate the lowest level first, then proceed to inoculate each higher level.

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7 and Instruction 348). Prepare materials. Test positive control compound in every experiment. Refer to Protocol 10 or Instruction 361 for instructions on randomization. Record deaths daily.

Day 1: Check Cultures. Discard experiment if contaminated. Weigh animals. Treat as instructed. Administer test agent based on initial average group weight.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Administer test agent.

Day 9: Administer test agent.

Day 13: Administer test agent.

Day 17: Toxicity day.

Day 18: Control early-death day. Weigh Day 2. Check s.c. tumors of control animals and record number without tumors as comment.
Day 48: Control no-take day.
Day 75: Final Evaluation Day.

QUALITY CONTROL: (refer to Protocol 7)

Schedule the positive control compound (NSC 409962* at doses of 12 and 6 mg/kg/injection) in every experiment, the regimen for which is Q4D x 4 beginning on Day 1. The lower T/C limit for the positive control is 150%. The acceptable untreated control median survival time is 29-40 days.

EVALUATION: (refer to Protocols 4 and 11)

The parameter measured is median survival time. Compute average animal body weights for Day 1 and Day 18, compute T/C for all test groups with > 65% survivors on Day 18. A T/C value < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

CRITERIA FOR ACTIVITY:

An initial T/C $\geq$ 125% is considered necessary to demonstrate moderate activity. A reproducible T/C value $\geq$ 150% is considered significant activity.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

*Positive control compound NSC 409962 is BCNU. CAS RN is 154-93-8.